3. Research Component Summaries

a. Remote Sensing of Greenbug and Russian Wheat Aphid Infestations

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In Phase I of the AWPM project, we conducted three independent field studies during the fall of 2001. These studies were also partially funded by a USDA-SBIR Phase I grant. Together, the studies demonstrated the feasibility of detecting greenbug infestations in winter wheat fields using a commercially available multispectral imaging system called the SST CRIS Crop Reflectance Imaging System (SST CRIS).

In the first study, we artificially infested two 3x3-m plots in a 0.4-ha wheat field with large numbers of greenhouse-reared greenbugs in early October, 2001. The objective of the study was simply to determine if we could visually identify the greenbug infested plots in SST CRIS imagery of the field. SST CRIS imagery was obtained using a Cesna-172 aircraft with the SST CRIS mounted vertically inside the fuselage of the aircraft. The field was imaged at approximately biweekly intervals from two weeks after infestation of the plots with greenbugs until the plots could be easily detected visually in SST CRIS imagery. By the second over-flight on October 25, 2001, the infested plots were visible in the SST CRIS imagery, and by the third over-flight on November 6, 2001 they were very visible (Figure 1). By November 6, the injury was clearly visible with the naked eye of a person standing near the plots as yellowed areas in the wheat field. While the results were encouraging they did not confirm that there would be a distinct advantage to using the multi-spectral imagery acquired by SST CRIS compared to ordinary color photography for detecting greenbug injury to wheat.

In the second study, we determined if greenbug injury to wheat plants could be detected in a typical field situation. In that study, 80 1-m^2 plots in a 0.8 ha wheat field near Perkins, Oklahoma were artificially infested with greenbugs at varying levels. The greenbugs used for infesting plots were reared in a greenhouse on wheat plants growing in 6-in. diameter pots. Plots were infested with varying numbers of greenbugs, ranging from no greenbugs to all the greenbugs from the foliage from four 6-in. diameter pots. Approximately two weeks after infestation, we commenced imaging the field at approximately biweekly intervals using the SST CRIS imaging system mounted in the Cesna-172 (Figure 2). At approximately the same date of

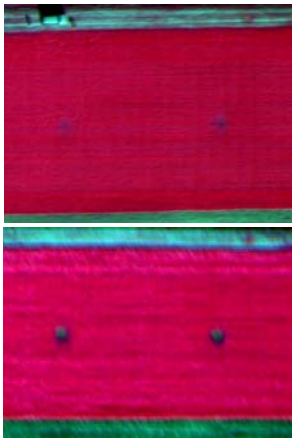

Figure 1. SST CRIS Crop Reflectance Imaging System imagery of an experimental wheat field near Perkins, Oklahoma for two dates in fall 2001: A) October 25, 2001 and B) November 6, 2001. Images adjusted to true reflectance.

each over-flight, greenbug density in each plot was determined by sampling 10 tillers from the plot and counting the number of greenbugs on each tiller. By the third sampling date (November 6, 2001) there was a statistically significant negative linear regression relationship between the normalized differenced vegetation index (NDVI) calculated from the red and NIR bands of SST CRIS normalized reflectance imagery and greenbug density (Figure 3). The existence of a statistically significant relationship between the density of greenbugs and NDVI in SST CRIS imagery clearly indicated that the injury caused by greenbug feeding on wheat plants could be detected using SST CRIS imagery. Furthermore, and most important, the injury was detectable at an earlier stage in its progression than could be detected by the human eye, because the plots were not obviously discolored by November 6 (the date the imagery was acquired). Greenbug densities in fields requiring insecticidal treatment to protect wheat yield typically range from 5 -

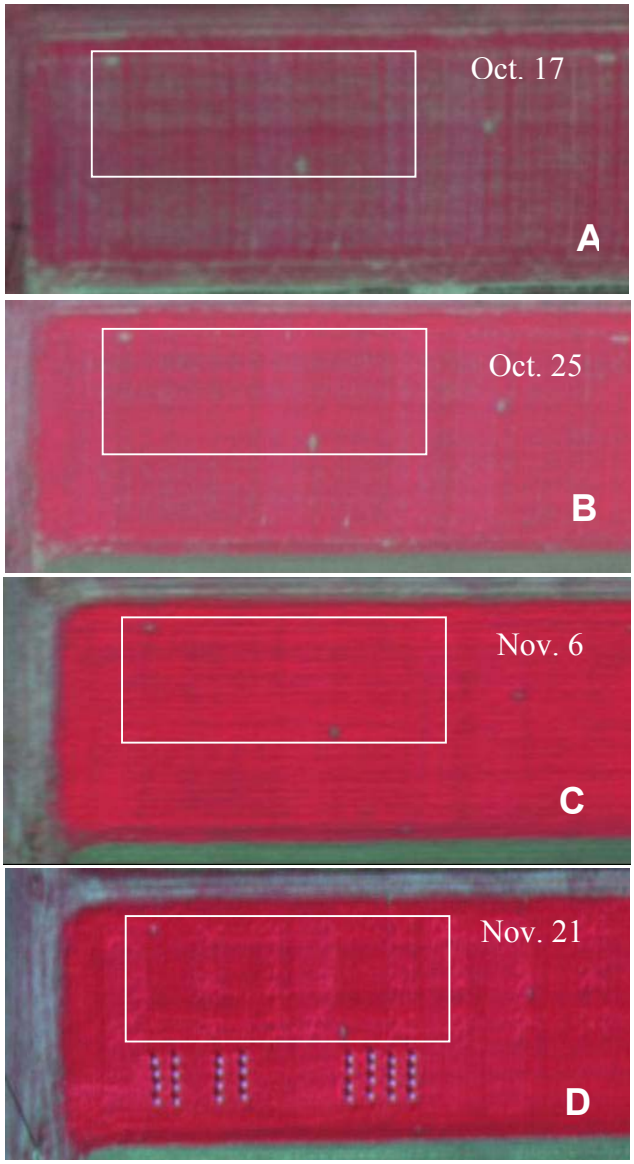

Figure 2. SST CRIS imagery of the area of a wheat field near Perkins, Oklahoma where 80 1-m^2 plots were infested with greenbugs. Plots with high greenbug density are vaguely identifiable in images C and D as small, darkened areas scattered throughout the white rectangle (which roughly indicates the area containing the 80 plots. All images were adjusted to normalized reflectance.

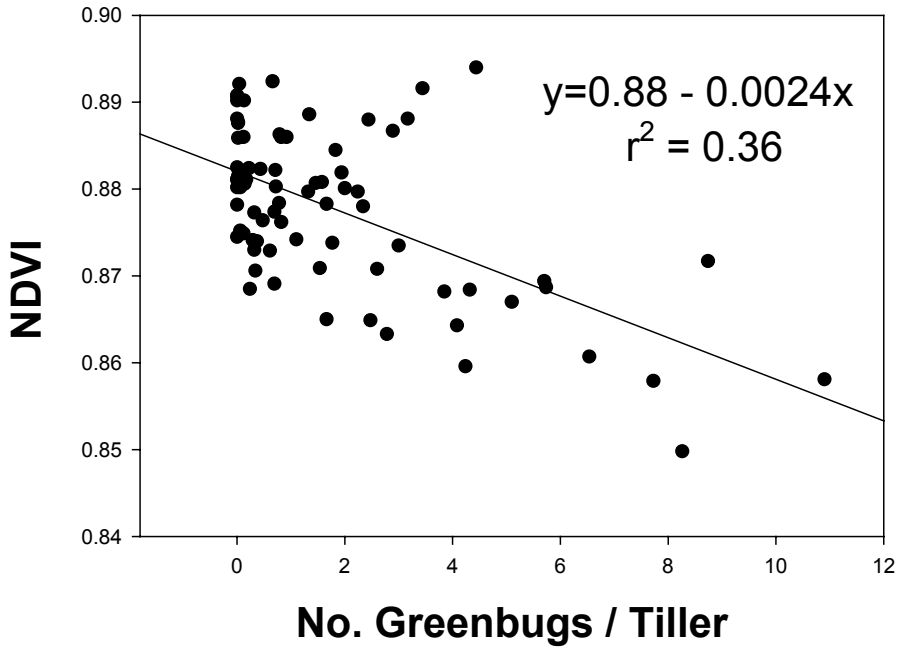

Figure 3. NDVI calculated from the NIR and red bands of an SST CRIS image acquired on November 6, 2001 versus the density of greenbugs in 1-m^2 plots in a wheat field near Perkins, Oklahoma.

12 greenbugs per tiller. Thus, an additional result of this study was verification that greenbug infestations could be detected using SST CRIS imagery at densities at or below those typically requiring insecticidal treatment.

The third study of the project was designed to determine if we could detect the spatial variation in greenbug density and plant injury in a wheat field naturally infested with greenbugs. To accomplish this study we scouted numerous wheat fields in Oklahoma during fall of 2001. Natural infestations of greenbugs were hard to locate during fall of 2001, but we were able to identify fields near Apache, Oklahoma where greenbug infestations occurred. In most of the fields, greenbug densities were in decline due to parasitism by natural enemies. We identified a field belonging to Mr. Paul Jackson where parasitism rates were low, and where consequently, the greenbug population would continue to increase in density. A drawback to the use of the field was that wheat plant growth in the field was highly variable for reasons other than the injury caused by greenbugs (Figure 4A). This heterogeneity made it very difficult to detect plant injury caused by greenbugs from other sources of heterogeneity in plant growth occurring within the field. We conducted an over-flight of the study field on December 18, 2001. The field was imaged from an altitude of approximately 1500 ft above ground level using an SST CRIS mounted in a Cessna 172 aircraft. At that altitude pixel size was approximately 13 x 13 cm. Several white cardboard panels were placed uniformly throughout the study plot prior to imaging, and the location of the center of each panel was determined using a global positioning system (GPS) instrument. The SST CRIS image was registered to the sampling grid using the known locations of the cardboard panels, which were clearly visible in the imagery.

Ground-based sampling was accomplished in the study field from December 18-20. A 95×95-m study plot was established in the field approximately one hour after the over-flight was completed. Sampling was undertaken at 400 pre-determined sample points arranged uniformly on the grid that spanned the 95×95-m study plot. Thus, sample points were 5-m apart within rows and columns of the grid. Wheat tillers were cut below the soil surface at three locations within 1-ft. of each of the 400 plastic stakes that marked the sample points (Figure 5). The tillers were placed in a plastic bag, labeled with the location of the stake, and returned to the laboratory

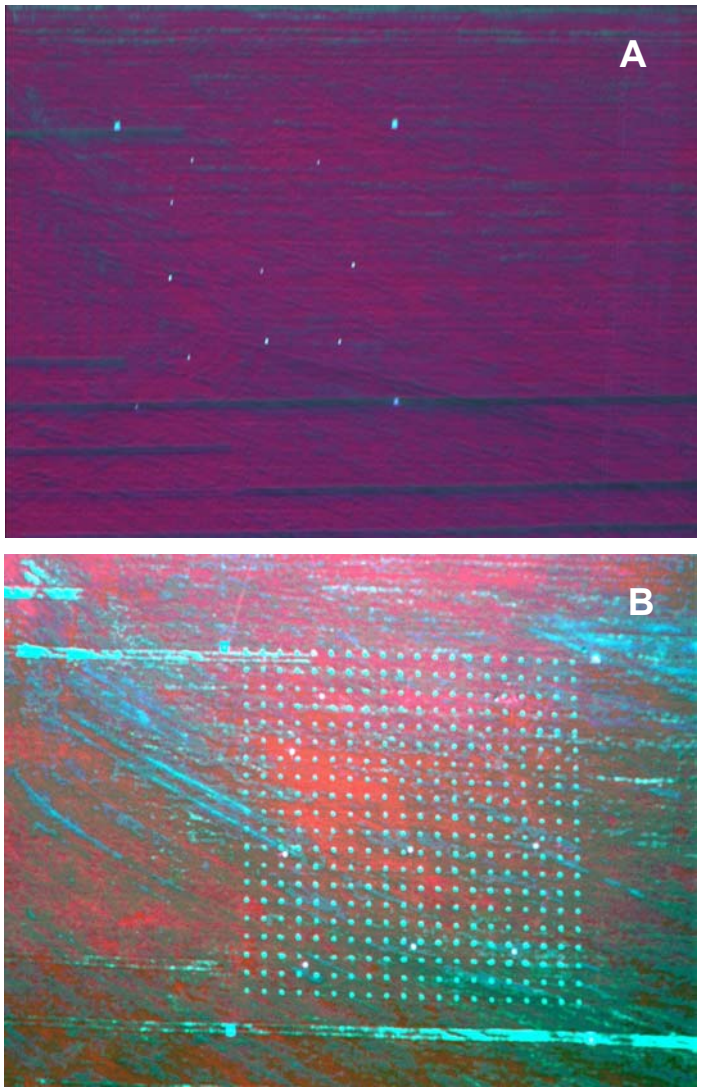

Figure 4. SST CRIS images of a wheat field near Apache, Oklahoma: A) image acquired December, 18, 2001, adjusted to normalized reflectance; B) image acquired February 5, 2002, not adjusted to normalized reflectance. Locations of white panels used as ground control points are visible in each image. Image 4b clearly shows the locations of the 400 sample points where wheat had been killed using herbicide.

where the greenbugs and number of tillers cut were counted and recorded.

Injury to wheat plants caused by greenbugs was rated at each sample point by cutting three tillers from different locations within 1-ft. of the plastic stake. Each tiller was rated according to the degree of greenbug-induced injury, using a damage rating system. According to the rating system, a tiller was given a rating of zero if no visual damage was present, and damage severity rating increased by integer values to a maximum value of 6 for a dead tiller. As a second measure of plant injury, relative wheat plant chlorophyll level was measured on each of the three tillers using a Spectrum CM-1000® hand-held chlorophyll meter.

A smaller study plot was superimposed on the 95×95-m study plot (Figure 5). The smaller plot was 25×25-m in size and included a total of 100 sample points arranged uniformly at distances of 2.5-m apart within rows and columns. Greenbugs, plant injury, and relative chlorophyll were measured using the same procedure as for the 95×95-m study plot.

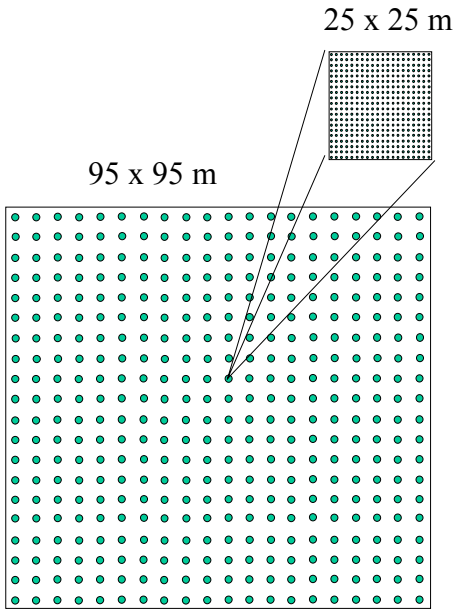

Figure 5. Plot design for ground-based greenbug, wheat plant damage, and relative chlorophyll sampling.

We evaluated reflectance patterns in the SST CRIS image using ERDAS Imagine 8.4© image analysis software. NDVI was calculated from the red and NIR wavelength bands of SST CRIS normalized reflectance imagery in order to visualize variation in reflectance patterns caused by spatial variation in greenbug density within the field. In order to ensure that we correctly located the pixels corresponding to the location of each sample point in the study plot, Roundup® herbicide was used to kill the wheat plants at each of the 400 sample points in the 95×95-m study plot after we had completed sampling. After the wheat plants had died, a second over-flight was made in which the field was imaged using the SST CRIS. White cardboard panels were placed uniformly throughout the study plot at the same locations as in the first over-flight. Areas of dead wheat plants were easily seen in the second image (Figure 4B). The centers of the white panels were used as ground control points for image registration. After registering the images, pixels for each band and for NDVI in the December 18, 2001 image centered on the location of the dead plants were extracted for categories of 3x3, 5x5, 7x7, and 9x9 pixels. The same procedure was used to obtain NDVI data for statistical analysis for the 25×25-m study plot. Mean NDVI was calculated for all 400 groups of pixels in each pixel number category for the 95×95-m plot and also for the 25×25-m plot. Pearson correlation coefficients for greenbug density, plant damage rating, and relative chlorophyll versus mean NDVI were calculated for data from the 95×95-m and 25×25-m study plots.

Correlations between NDVI and greenbug density, plant damage, and relative chlorophyll levelwere significant for all pixel groupings for the 95×95-m study plot (Table 1). Correlations of NDVI versus greenbug density and plant damage rating were significant for all pixel

groupings for the 25×25-m plot, but correlations of NDVI with relative chlorophyll were not significant for pixel groupings on the 25×25-m plot. In spite of significance, correlation coefficients were very small. We think that the high degree of heterogeneity in wheat plant growth within the study field was partially responsible for the small correlations. This statement is supported by the observation that correlations for greenbug density and damage rating for the 25×25-m plot were generally larger in magnitude than those for the 95×95-m plot. This probably occurred because less variability in wheat growth was encountered in the small area encompassed by the 25×25-m plot compared to that in the much greater area of the 95×95-m plot.

Table 1. Pearson correlation coefficients for greenbug density, relative chlorophyll level, and plant damage rating versus NDVI for an SST CRIS image of a wheat field near Apache, Oklahoma acquired on December 18, 2001.

NDVI	Greenbug Density	Relative Chlorophyll	Damage Rating
95-m^2 Study Plot			
3x3 pixels	**-0.25***	**0.20***	**-0.25***
5x5 pixels	**-0.25***	**0.21***	**-0.26***
7x7 pixels	**-0.25***	**0.21***	**-0.27***
9x9 pixels	**-0.26***	**0.21***	**-0.27***
25-m^2 Study Plot			
3x3 pixels	**-0.37***	**0.16**	**-0.31***
5x5 pixels	**-0.38***	**0.16**	**-0.30***
7x7 pixels	**-0.38***	**0.16**	**-0.30***
9x9 pixels	**-0.37***	**0.16**	**-0.30***

* Correlation differs significantly from zero ($P<0.01$).

Another factor accounting for the small correlations was the sampling processes used to estimate greenbug density, plant damage rating, and relative chlorophyll. Even though the sampling methods were very time consuming, they were fraught with very high sampling errors, and in the case of estimates of greenbug density, bias that occurred among the five individuals that counted the samples. We believe these factors seriously reduced the evidence of the true strength of the relationship between NDVI and the measures of greenbug population density. Use of less error prone methods for ground-based sampling would have resulted in stronger correlations. However, it is very important to note that even under very poor circumstances we were able to document a relationship between NDVI in SST CRIS normalized reflectance imagery and the three measures of spatial variability of greenbug density within a wheat field.

There was no decrease in correlation of NDVI with greenbug density, plant damage, and relative chlorophyll as pixel grouping increased in size from 3x3 to 9x9 pixels (Table 1). This suggests that patches of greenbugs of various densities within a field occur at a scale larger than the ca. 1.15 x 1.15-m area of the largest pixel grouping we created. This result indicates that patches within a wheat field with varying densities of greenbugs are large enough to be detected in SST CRIS imagery even if pixel size was as large as 1-m^2.

Summary

We demonstrated through the series of experiments that: 1) Areas of greenbug infested wheat within a field can easily be distinguished from healthy wheat in a false color composite image of green, red, and near infrared (NIR) bands of normalized reflectance SST CRIS

imagery; 2) There is a strong negative linear relationship between greenbug density and NDVI calculated using the red and NIR bands of normalized reflectance SST CRIS imagery; 3) Greenbug infestations can be detected using NDVI calculated from SST CRIS imagery at densities below typical treatment thresholds; and 4) Spatially variable greenbug infestations in a wheat field can be differentiated in an NDVI image calculated using the red and NIR bands of normalized reflectance SST CRIS imagery. These four results provide strong evidence that remote sensing using the SST CRIS can be used to detect greenbug infestations in wheat fields before insecticide application would typically be required to protect the crop from economic losses. Furthermore, the project laid a firm foundation for future research to develop methodology to detect infestations of greenbugs at both the whole field and sub-field levels using the SST CRIS imaging system, from which we believe we can develop an operational greenbug detection system.

b. Natural Enemy Dynamics in Diversified Cropping Systems

Prepared by Mpho Phoofolo

Introduction

A research component of the AWPM project was to unravel details of the dynamics of aphid natural enemies within diversified cropping systems compared to mono-cultural wheat only cropping systems in order to be able to better predict the effects of particular cropping system configurations on biological control of greenbugs and Russian wheat aphids in wheat agroecosystems. During the summer and autumn of 2003 plans were developed and research was initiated to address this problem. Both field and laboratory studies were deemed necessary to unravel the complexities of how predators utilize prey in complex wheat agroecosystems compared to simple, wheat only, systems.

The overall objective of the laboratory study is to test the potential applicability and robustness of stable isotope analysis to insect predator-prey trophic interactions in an environment where most of the contributing factors can be manipulated and/or controlled. In other words, the study is aimed at developing a set of standards against which the use of stable isotope analysis in the field can be based. Specifically, the study is designed to:

1. determine the relationship between the $\delta^{13}C$ and $\delta^{15}N$ in insect predators relative to ratios in the aphid prey and host plants;
2. assess the isotopic turnover rate/time in predators relative to diet changes (aphid species);
3. test the performance of the linear mixing models in reconstructing the diets of aphidophagous insect predators.

The objective of field studies is to determine why natural enemies of aphids are more abundant in diverse versus simple wheat dominated landscapes and diverse versus simple wheat dominated within-farm cropping systems. More specifically, we seek to determine how the mix of crop and non-crop vegetation influences populations and communities of natural enemies at landscape and field scales. The paragraphs that follow outline the laboratory and field research that we designed and initiated to address these issues.

i. Methods for Laboratory Research on Natural enemy Dynamics

Relationship between $\delta^{13}C$ and $\delta^{15}N$ in predators relative to ratios in aphid prey and host plants.

The leaf tissue from each plant species (alfalfa, sorghum, and wheat) will be collected and then freeze-dried to make three 20 mg samples per species for isotopic analysis. To determine whether different aphid species from the same host plant have the same or different isotopic signatures, several aphids of each species in Table 1 will be collected into glass vials and dried to make four 10 mg samples.

Table 1. Aphid species for determining taxon-specificity in isotopic signatures.

Host plant	Aphid species
Alfalfa	A. pisum, A. kondoi, and T. maculata
Wheat	S. graminum, D. noxia, and R. padi
Sorghum	S. graminum and R. maidis

To determine taxon-specificity in isotopic signatures among the predator species with the same feeding history, two sample populations of lady beetle larvae from each of the most commonly occurring species in (central) Oklahoma annual crops (i.e., Hippodamia convergens, Coleomegilla maculata, Coccinella septempunctata) will be reared from 1st instar to pupal stage. One population of each species will be fed pea aphids (or any one of the available aphid spp. from alfalfa) and the other population fed greenbugs. Upon becoming adults, 10 beetles (5♀ and 5♂) will be randomly selected from the population and frozen within 24 hours post-emergence for isotopic analysis before any adult feeding takes place.

Isotopic turnover rates.

Isotopic turnover rates will initially be determined only on H. convergens. If results on taxon-specificity in isotopic signatures among lady beetles show significant differences among species, turnover rates in C. maculata and C. septempunctata will be determined later. H. convergens adults will be obtained from the sample population reared from 1st instar to pupae on pea aphids. Within 24 hours of becoming adults beetles will be randomly subdivided into 4 groups (treatments): (i) the control group, which will continue on the same aphid prey as the larvae, (ii) the group which will be switched to a diet of greenbugs, (iii) the group switched to a mixed diet of greenbugs and pea aphids (in constant pre-determined proportionate amounts), and (iv) the group switched to a mixture of greenbugs, corn leaf aphids, and pea aphids (in constant pre-determined proportionate amounts).

Subsets of 8 beetles (4♀ and 4♂) will be selected from group (i) on the 10th, 20th, and 40th day post adult emergence, held with water but no food for 24 hours (to allow emptying of food material from their guts) before being frozen for isotopic analysis (fewer sub-samples because no turnover is expected in this group). To determine the isotopic turnover rates in beetles that switch diets (i.e., groups (ii) to (iv)), subsets of 8 beetles (4♀ and 4♂) will be serially selected from each group, held with water but no food for 24 hours then frozen for isotopic analysis. This sub-sampling will be done every other day for the first 10 days post emergence and thereafter on the 14th, 20th, 28th, and 40th day post-emergence. Sub-sampling is more frequent at the beginning of the experiment so as to accurately determine the turnover pattern/trend as well as the half-lives of the (isotopic) elements in the selected predator tissues.

Two separate isotopic analyses will be performed on each individual, one on the forewings (elytra) and the other on the remaining body (i.e., w/o elytra). The elytra are analyzed separately because they are made of materials that are believed to become rather metabolically inert following wing synthesis and therefore are not expected to show rapid isotopic turnover for beetles that switch diets after reaching adult stage. Therefore, the working hypothesis is that stable isotope analysis on the elytra will reveal the larval diet histories of individual beetles whereas the analysis on the body will reveal the adult diet history (i.e., recent feeding record).

Sample preparation for isotopic analyses

Prior to being sent for isotopic analysis all the samples (of plants, aphids, and predators) will be either oven-dried or freeze-dried, ground into fine talcum powder consistency using a ball mill, and then sealed into 5 x 9 mm tin capsules. These capsules will then be shipped to any of the laboratories that will be chosen to do the stable isotope analyses.

Reconstruction of proportionate contributions of diet sources by the linear mixing models

Once the $\delta^{13}C$ and $\delta^{15}N$ for plants, aphids and lady beetles are known proportionate contributions of different (aphid) species to the predators will be determined by manual calculations using equations (3a) and (3b) for predators that were fed a mixed diet of two aphid species and equations (5a), (5b), and (5c) for predators fed a diet of three aphid species. Results from manual calculations will be confirmed by using the Excel spreadsheet program made available at http://www.epa.gov/wed/pages/models.htm for performing calculations for both a two-source model and a three-source model.

Data analysis

Data will be subjected to various appropriate statistical tests.

ii. Methods for Field Research on Natural enemy Dynamics

1. Wheat

Sampling in fall and spring with sampling frequency per plot based/dependent on levels of aphid infestation.

Instead of (VS):

Sampling during each of the 5 phenological/developmental wheat stages (i.e., tillering, stem elongation, boot, head emergence-flowering, and the soft dough stage).

a. Aphid pests: -
    - Determine plant growth stage
    - Divide each plot into 2 (east and west) subplots
    - Randomly collect 50 tillers per subplot by traversing each subplot and picking 10 tillers at approximately every 30 feet (Giles et al 2000).

b. Predators (coccinellids, chrysopids, anthocorids, nabids, syrphids, carabids, staphylinids, and spiders)

All predators will be sampled using the following combination of methods:

1. Random placement of 0.5 m^2 quadrats in 3 random locations per subplot
2. Sampling of each quadrat for 1.5 minutes with a suction sampler
3. Immediate careful ground searching and collecting of predators in the area just vacuumed (may exclude depending on suction sampler performance)

- Density is estimated by pooling counts from suction sampling and visual ground search

2. Sorghum

Sampling done during each of the following 5 phenological/developmental sorghum stages (stage 2 = collar of fifth leaf visible, stage 3 = growing point differentiation, stage 4 = final leaf visible in whorl, stage 5 = boot stage, and stage 6 = half bloom).

(a) Aphid pests: - Greenbugs and other aphids will be sampled similarly regardless of the plant developmental stage.

- Determine plant growth stage
- Count aphids on 10 randomly chosen plants
- Count plants in two 1.0-m sections of row

OR

- Count aphids in all plants in 1 m of row in 4 randomly chosen locations

(b) Predators – use one of the following:

- Visual counts per plant (Kring et al 1985; Tyler et al 1974; Lopez & Teetes 1976) on 10 plants
- Visual counts on all plants in 1 m of row from four randomly chosen locations (Parajule et al 1997)
- Quadrat sampling involving counting all predators trapped within a 0.5 m^2 or 1.0 m^2 area from 3 or 4 random locations per plot (Michels et al 1996)
    - Maybe combined with suction sampling

3. Alfalfa and cotton

- Purpose of predator and prey density estimates from alfalfa and cotton?
    - Correlation with predator density in adjacent intercrops
    - Within crop comparisons across years

Monitoring/sampling in alfalfa

- Aphid density/abundance determined by stem sampling (25 stems per subplot)

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- Predator density/abundance determined by suction sampling as described above for wheat sampling; plus pitfall and sticky traps as described below.

Monitoring/sampling in cotton

- Sampling to span from the time the plants first true leaves to early open ball stage
- Visual count of aphids and predators on plants in 1 m of row in four randomly chosen locations per plot.
- Use of pitfall and sticky traps?

Dispersal and activity density

(a) Use of PCR based Stomach Content Analysis (genetic markers)

i. PCR primers already existing for cereal aphids
ii. Possibility of developing PCR primers for aphid spp. in:
1. alfalfa e.g. pea aphid, blue alfalfa aphid
2. cotton ?
3. Do similar studies as that of Chen et al (2000)need to be made for different predator groups [just like done by Greenstone & Shufran (2003)

(b) Use of Stable Isotopic Analysis

(i) Carbon isotopic signatures
(ii) Nitrogen isotopic signatures

(c) Use of traps (pitfall and sticky)

Pitfall traps

Each plot of wheat (both in diversified and monoculture) will have 4 traps set up at random (permanent) locations. Guides (14 x 122 cm galvanized sheet metal strips) will be used to enhance trap capture efficiency and will be arranged such that 2 traps will have guides facing the alfalfa plot and 2 facing away from alfalfa. To compare predator abundance and activity between adjacent crops 2 sets of paired traps will also be set up simultaneously in the alfalfa plots. Predators caught on traps will be counted and removed every week.

Sticky traps

- Yellow Pherocon® AM sticky traps will be used
- Each trap will be mounted (stapled) on wooden stakes (2 feet above ground) so that the trap has two surfaces, east-facing and west-facing

Sticky trap arrangement per plot

- Each plot (of wheat (diversified and monoculture), alfalfa, sorghum, and cotton) will be subdivided into 2 subplots (east and west)
- 3 traps will be set up at random locations along a north-south direction in each subplot.
- Predators caught on traps will be counted every week and the traps will be replaced every other week.

Trapping (both pitfall and sticky) will be shifted from alfalfa/wheat to other crops as shown in the following Table:

Trapping period	Adjacent crops
Fall-winter-spring	Alfalfa and wheat
Spring-summer	Wheat and sorghum
Summer-fall	Sorghum and cotton
Fall	Cotton and alfalfa